

Polycyclic Aromatic Hydrocarbons (PAHs) and Trace Metals in Some Brands of Sausage Roll in the Nigerian Markets

Olutona, Godwin Oladele*⁺; Arigbedede, Olayemi Elizabeth; Dawodu, Modupe O.
*Industrial Chemistry Programme, College of Agriculture, Engineering and Sciences, Bowen University,
Iwo, NIGERIA*

ABSTRACT: Concentrations of PAHs and trace metals in five selected brands of sausage commonly available in the Nigerian market were determined in this study. The health risk assessment of both PAHs and trace metals was also evaluated. Five different brands of commercially available sausage rolls were purchased from various retail shops in Ibadan, Ijebu Ode, and Iwo markets in Nigeria. Methods: PAHs extraction of the sausage roll samples was carried out with a Soxhlet extractor and clean-up of the extract was done using activated silica gel. The analysis of target analytes including the 16 priority PAHs was performed with Gas Chromatography equipped with Flame Ionization Detector (GC-FID), while the concentrations of trace metals were determined using Atomic Absorption Spectrophotometry (AAS) after acid digestion of the sausage samples. The concentrations of Σ 16 PAHs in sausage samples were in the range of 12.5-36.2 $\mu\text{g/g}$. Benzo (a) pyrene was predominant in all the samples investigated and their concentrations were above 0.001 $\mu\text{g/g}$ limit in processed cereal-based food products as stipulated by the European Commission Regulation. The brands of sausages were mainly polluted with 4- and 5-ring PAHs. The order of increase of PAHs in brands of sausage roll was: SB > YM > GA > BG > RT. The trace metals concentrations (mg/kg) ranged as follows: Zn (1.01-71.0), Cu (ND-8.12), and Cr (ND-16.4). Cd was not detected in all the samples analyzed. Pb ranged from 2.34-13.0 mg/kg infringing the safe limit for cereals and cereal-based food products of 0.3 mg/kg and 0.05 mg/kg as stipulated by the FAO/WHO and European Commission, respectively. Conclusion: This study revealed the occurrence of PAHs and trace metals at varying concentrations in the sausage samples analyzed. Hence, it is imperative to constantly monitor and control the concentrations of these pollutants in sausages since they are usually consumed by school children and youths.

KEYWORDS: Toxicology; Contamination; Sausage roll; Health risk assessment; Public health.

INTRODUCTION

A sausage roll is described as a cylindrical meat product usually produced from ground meat, most times

pork, beef, or veal, alongside salt, spices, flavorings, and breadcrumbs covered with a sheet of puff pastry around

* To whom correspondence should be addressed.

+ E-mail: delog2@gmail.com

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it and afterward baked. The sausage roll is a ready-to-eat food product and very affordable, tasty, very easy to digest, compact in size, and can be preserved for a long time. It is a common snack enjoyed by all age groups and social classes.

Polycyclic aromatic hydrocarbons are produced as a result of incomplete combustion and pyrolysis processes from anthropogenic and natural sources of carbonaceous materials such as wood and coal. Food processing such as roasting, grilling, smoking, and drying also significantly contribute to the source of PAHs in food substances [1-3]. Studies have shown that ashes produced from firewood as a result of the smoking and roasting of food contain an appreciable amount of PAHs [4-5].

Oils and fats from vegetables are important dietary intake of PAHs which can be introduced through a direct method like vegetable oils utilized for flavoring and margarine which can be applied to food while cooking. They can also be introduced through the indirect method of cooking that incorporates fats into foods, e.g. in cereal-based items, pastries, cakes, and biscuits [6]. Recent research on exposure to PAHs shows that food items are the primary cause of human exposure to PAHs and cereals are known to be the most important sources [7].

Some PAHs are identified to demonstrate both cancer-causing and mutagenic characteristics. Nevertheless, PAHs that do not exhibit carcinogenic properties, synergistically promote the carcinogenic properties of different PAHs. Benzo[a]pyrene is carcinogenic and it has been used by many authors as a proxy to understand the influence of diet on the consumption of polycyclic aromatic carcinogens [8]. *Alomirah et al.* (2011) [9] discovered that the human dietary exposure to Benzo[a]pyrene was 2-500 ng/day which is above exposure through inhalation of 10-50 ng/day. PAHs can cause cancer not only through the mutagenic properties of their metabolites, which can initiate tumor formation by modifying DNA, but also through their parent compounds, which enhance tumor progression [10]. A particular PAH like naphthalene when inhaled or ingested in high concentration can result in the breakdown of red blood cells. Anthracene and Benzo[a]pyrene have also been discovered to affect the skin, that is, bring about an allergic reaction in the skin of animals and humans [11].

In the previous two decades, the levels of PAHs have increased owing to the development and creation of new

industries, particularly in developing countries resulting in increased amounts of PAHs in food products. However, the awareness of the populace concerning the dietary route of exposure to PAHs has grown exponentially in this new age due to their identified toxicity in food and the environment. Consumption of baked and packaged flour-based products like sausage rolls has progressively increased especially in Nigeria particularly because they are a convenient food product and generally affordable by all classes of people and yet established maximum, allowable levels for PAHs remained unset. Therefore, it is imperative to identify the dietary exposure of PAHs by describing the connection between the amounts incorporated in sausage rolls along with their nutritional tendencies [12].

Apart from PAH contaminations, heavy metal contamination may arise from the materials used in sausage rolls baking. Heavy metals may pollute food items via agricultural technology, industrial contamination, environmental sources, and the processing of food. Metals recognized in food may be nutritionally essential or extremely poisonous based on the nature of the metal and its level. Metals such as Zn, Fe, Se, Mo, and Cu are present in small concentrations which may be bio-toxic to humans if it is sufficiently available [13-15]. Pb and Cd are deleterious to humans because they are toxic and cannot be tolerated even at low concentrations [16]. The occurrence of Cadmium in food is poisonous to human well-being and the adsorption of a couple of milligrams can lead to several serious diseases or neoplasia. Likewise, Pb in food has toxic effects on adults as well as young children. There are reports that indicate Pb levels in the blood as little as 10 mg/dL are very unsafe for developing children [17].

In light of this, heavy metals have become a focus of public interest since the adverse health effects of these metals are now recognized and accurate analytical methods have ensured the possibility to identify metals notwithstanding its minute concentration, down to the thousandth of mg/kg and also international legislations are now developed for trace elements as contaminants in food. In this present stage of industrialization and development, the concentration of heavy metals in foodstuff *via* ingestion is required for the dietary intake of trace metals from food consumption. Therefore, continuous research should be undertaken to detect the levels of certain trace metals in food, either for their essential nature or toxicity.

It is quite important to do so since some of the essential ones at elevated levels have serious adverse health effects. Generally, trace metals that are not easily degradable or metabolized and are usually persistent may be biologically accumulated in food items trapped on the outer surface or may be added due to the manufacturing or processing of food for consumption [18].

Recently, the information available on polycyclic aromatic hydrocarbons and trace metals in sausage rolls are scanty. It is in line with this assertion that this study is designed to reveal some trace metals and PAHs content of some sausage roll brands commonly available in Nigerian markets. Furthermore, the study also seeks to evaluate the Dietary Daily Intake (DDI) and the health risk assessment of PAHs and trace metals from the consumption of sausage rolls. This will help in assessing the safety of this food item and its appropriateness for human consumption. It will also show the level at which the brands studied to comply with the recommendations provided for concentrations of PAHs and trace metals.

EXPERIMENTAL SECTION

Sampling and sample preparation

A total of five different brands of sausage rolls commercially available were purchased from various retail shops in Ibadan, Ijebu Ode, and Iwo markets in Nigeria. In selecting these samples, precautions were made in order to reflect the various popular brands consumed by different income groups and different age groups, especially children. It was also influenced by the availability of sausage products at the time of the study.

The sausage roll samples were cut open from their polyethylene wrappers. Then the sausage roll samples were chopped into smaller bits and homogenized. The samples were preserved in the refrigerator prior to analysis.

Reagents and Chemicals

All reagents and chemicals used were of analytical grades. These include: 69.5-70.5% Nitric acid (BDH, England), 70% Perchloric acid (Sigma-Aldrich, Germany), Hydrogen peroxide (BDH, England) 99.5% Acetone (BDH, England), 5% water content silica gel (Mesh size: < 45 μm) (Merck, Germany), anhydrous sodium sulphate (VWR, England) and 96.5% n-hexane. The working standard of Zn, Pb, Cu, Cr, and Cd were prepared by diluting concentrated stock solutions of 1000

mg/L (Chem-Lab NV, Industrienterrein, Belgium) with 2% nitric acid. Distilled water was used for the dilution of samples and rinsing glassware and sample bottles.

Trace Metals Analysis

One g of each sample was weighed into a beaker. Thereafter, 10 mL of HNO_3 acid was added and the beaker was placed in the fume cupboard on a hot plate. The contents were replenished with HNO_3 until the fume was colorless to avert the content from drying up. The samples were allowed to cool for 5 min, and 1 mL each of HClO_4 acid and H_2O_2 were added thereafter. The sample was further digested for about 15 to 30 min. The digested sample was then transferred into a 25 mL volumetric flask and made up with distilled water to mark. A blank determination was carried out to establish blank levels for the metal analysis.

The concentrations of Zn, Pb, Cu, Cr, and Cd in digested sample solution were determined using Flame Atomic Absorption Spectrophotometer (FAAS, AA990 PG instrument, UK) available at the Central Laboratory, Bowen University, Iwo. Standard solutions and analytical blanks were analyzed in the same manner as the samples and the equivalent concentrations of each sample were recorded.

PAHs Extraction, Clean-up, and GC-FID Analysis

For the extraction of PAHs, 300 mL of the solvent (n-hexane) was poured into the flask. 20 g of each sample were extracted for three and half hours to complete one extraction. The extract was preserved in a refrigerator prior to GC-FID determination.

Clean-up was performed using a 30 mm column packed with silica gel. The clean-up process is basically to remove all other forms of impurities which might be present in the eluate. The recovered eluate after clean-up was evaporated to dryness and then reconstituted with 1 mL of n-hexane and stored in vials.

Isolation, identification, and quantification of PAHs which follows a standard procedure of organic extraction, sample clean-up, and analysis using Agilent gas chromatography model 7890A equipped with Flame Ionization Detector (FID) were done at the Central Laboratory, National Institute of Oceanography and Marine Research (NIOMR), Victoria Island, Lagos, Nigeria. The system was fitted with column HP5 (30m x 320 μm x 0.25 μm). 1 μL aliquot of prepared sample

extract was injected into the column in splitless mode at an injector and interface temperature of 270°C. The split flow rate was 1.2 mL/min while the oven temperature was the program was at 60°C and later increased to 210°C at 12°C/min and 320°C at 8°C/min held for 5 min, the total run time was 32.25 min, and the detector temperature was 325°C. The Agilent 7890A GC ChemStation software was used to obtain the chromatogram. Identification of PAHs in the sample was focused on the comparison of the retention times of the peaks with those obtained from a standard mixture of PAHs (standards supplied by the instrument manufacturer). Quantification was based on the external calibration curves prepared from the standard solution of individual PAHs and the corresponding areas of their respective chromatogram. The limits of detection (mg/L) were assessed three times on the basis of background noise produced from the analysis of blank samples (n=3). The Limit of Detection (LOD) and Limit of Quantification (LOQ) were the levels of analyte that provided a signal-to-noise ratio of 3 and 10, respectively.

Quality Control

In order to guarantee the accuracy of results, appropriate handling of samples was applied to prevent environmental contamination. Equipment and containers for preserving the samples were cleaned thoroughly to avoid cross-contamination during sample collection and preparation. A standard solution of the analyte-containing seventeen PAHs compounds was prepared. Working standards were prepared by diluting with hexane. Silica gel (Mesh size: < 45 µm; Merck, Germany) was heated at 300°C in an oven for one hour, preserved in an airtight desiccator, and reactivated at 150°C for 30 min immediately prior to use. The LOD and LOQ (mg/kg) were: Zn (0.003:0.03) Pb (0.013:0.13), Cu (0.004:0.04) Cr (0.005:0.05) and Cd (0.003:0.03), respectively. Blank determination for trace metal was performed and this was carried out by running a separate determination under the same experimental conditions applied in the actual analysis of the sample but excluding the sample. The AAS was re-calibrated for every batch of 10 samples analyzed by analyzing a reagent blank. The recovery analysis was carried out to determine the accuracy of the analytical methods employed in this research and this was done using the spike recovery method. The values from the recovery analysis for all the trace metals studied were higher than 90%.

Health Risk Assessment of Polycyclic Aromatic Hydrocarbons

Estimation of Benzo[a]pyrene Equivalent (BaPeq)

Benzo[a]pyrene was considered the greatest effective cancer-causing PAH after Dibenz[a,h] anthracene. Consequently, the total PAH content is expressed as BaPeq to show the toxic potency [9]. The BaPeq of sausage roll was calculated according to Eq. (1) [12]:

$$BaPeq_i = C_{PAH_i} \times TEF_{PAH_i} \quad (1)$$

Where,

C_{PAH_i} = Concentration of PAH congener i in food (µg/kg)

TEF_{PAH_i} = Toxic Equivalent Factor of individual PAH as stipulated by Nisbet and Lagoy [19]

The $\Sigma BaPeq$, i.e the carcinogenic potencies of 16 PAHs were calculated as the sum of each individual BaPeqi.

Estimation of Dietary Exposure

The consumption rate of 80 g per day was used for the estimation of dietary intakes of PAHs in sausage rolls [22]. The calculation of dietary exposure was estimated for BaP, PAH2, PAH4, PAH8, Σ 16PAHs, and Σ BaPeq by multiplying concentrations for each sausage roll sample by the consumption rate (CR) and by dividing the dietary intake with the average body weight of an adult (70 kg), youth (65 kg) and school child (35 kg). The equation is as follows [20]:

$$EDI = \frac{C \times CR}{BW_{average}} \quad (2)$$

Where,

EDI = Estimated daily intakes (µg/kg bw/day).

C = Concentration of BaP or PAH2 or PAH4 or PAH8 or Σ 16 PAHs or Σ BaPeq in the biscuit sample (µg/g).

$BW_{average}$ = Average Body Weight (kg/person).

Marging of Exposure (MOE)

The formula below was used to estimate the MOE for all scenarios [6]:

$$MOE = \frac{BMDL_{10}}{EDI} \quad (3)$$

Where,

MOE = Margin of Exposure ($\mu\text{g}/\text{kg}$ bw/day)

Where,

$BMDL_{10}$ = Benchmark Dose Lower Limit (mg/kg bw/day)

EDI = Estimated daily intakes ($\mu\text{g}/\text{kg}$ bw/day)

BaP Toxic Equivalent (TEQ)

The BaP mutagenic equivalent (BaP_{MEQ}) for each PAH was estimated by the following formula:

$$BaP_{MEQ} = \sum C_i \times BaP_{MEF} \quad (4)$$

Where,

BaP_{MEQ} = Mutagenic Potency relative to BaP

C_i = Concentration of individual PAH ($\mu\text{g}/\text{g}$)

2.6.5 Incremental life Cancer Risk (ILCR)

The ILCR for all scenarios studied caused by PAHs dietary exposure in brands of sausage roll was estimated using the formula below:

$$ILCR = \frac{EDI \times EF \times ED \times SF \times CF}{BW \times AT} \quad (5)$$

Where EDI is the estimated daily intake, EF is the exposure frequency (365 days/year), ED is the exposure duration (year) (adult=55.2 years established on the Nigerian life expectancy which relates with the information from WHO announced in 2018. In this work, the exposure duration for a schoolchild and youth is assumed as 15 years and 25 years respectively. SF is the oral cancer slope factor of BaP (7.3 $\text{mg}/\text{kg}/\text{day}$), CHY (0.0073 $\text{mg}/\text{kg}/\text{day}$), NAP (0.119 $\text{mg}/\text{kg}/\text{day}$), BaA (0.73 $\text{mg}/\text{kg}/\text{day}$), BbF (0.73 $\text{mg}/\text{kg}/\text{day}$), BkF (0.073 $\text{mg}/\text{kg}/\text{day}$), DahA (7.3 $\text{mg}/\text{kg}/\text{day}$) and IndP (0.73 $\text{mg}/\text{kg}/\text{day}$) [21]. CF is the conversion factor (10^{-6} mg/ng), BW is body weight (kg), AT is the average lifespan for carcinogens (days) i.e $ED \times 365$ (20148 for adults; 9125 for youths; 5475 for school children).

Estimated Daily Intake (EDI) for trace metals

The daily intake of trace metals was estimated to evaluate the average daily concentration of metal in the body system of a particular bodyweight consumer. The estimated daily intake of metals has been extensively evaluated by authors [20, 22, 23].

$$EDI = \frac{C_{metal} \times D_{food\ intake}}{BW_{average}} \quad (6)$$

EDI = Estimated daily intake ($\mu\text{g}/\text{kg}$ bw/day).

C_{metal} = Concentration of heavy metal in the biscuit sample (mg/kg).

$D_{food\ intake}$ = Daily food intake ($\text{kg}/(\text{person}\ \text{day})$)

$BW_{average}$ = Body weight (kg/person)

Target Hazard Quotient (THQ)

Target hazard quotients were created by United State Environmental Protection Agency to assess possible health effects related to chronic exposure to contaminants [23]. The THQ values via the ingestion of sausages by school children, youths, and adults were assessed for each trace metal with the following formula [24]:

$$THQ = \frac{EF \times ED \times C \times DFI}{RFD \times BW \times AT} \times 10^{-3} = \frac{EDI}{RFD} \quad (7)$$

Where EF is the exposure frequency (365 days/year), ED is the exposure duration of 55.2 years based on the Nigerian life expectancy rate for an adult, C is the concentration of trace metals in sausage roll samples (mg/kg), DFI is the daily food intake ($\text{kg}/\text{person}\cdot\text{day}$), RFD is the oral reference dose for the metals under study (Cd (0.001); Pb (0.004); Cu (0.04); Zn (0.3); Cr (0.003) [22], BW is the body average weight (kg) and AT is the average exposure time for non-carcinogen (days) ($365 \text{ days}/\text{year} \times ED$) [22], 10^{-3} is the conversion factor. If the target hazard quotient is < 1 , it indicates no health concern [22-23].

Statistical analysis

The invariant treatment of data was carried out by one-way analysis of variance (ANOVA) to discover the variations between the means of samples at 95% confidence level and to identify precisely which brand varies significantly from the other brands. The statistical calculations for mean and standard deviation for concentrations of PAHs and trace metals from triplicate sampling were performed with Statistical Package for Social Sciences IBM SPSS® software version 20.0 for window evaluation. Duncan's multiple range tests were carried out post hoc to describe the differences between brands. The results of the mean values of PAHs and trace metals concentration were employed for the evaluation of health risk assessment.

RESULTS AND DISCUSSION

Polycyclic aromatic hydrocarbons

The concentration of polycyclic aromatic hydrocarbons

Table 1 presents the mean concentrations of sixteen compounds of PAHs detected in five brands of sausage roll samples. The total mean concentration of PAHs ranged from 12.5 $\mu\text{g/g}$ in RT to 36.2 $\mu\text{g/g}$ in SB. The order of increase of PAHs in brands of sausage roll was: SB > YM > GA > BG > RT. The distribution of several forms of PAHs in terms of their number of rings in the sausage roll samples analyzed follows the order: 5-ring > 4-ring > 6-ring > 3-ring > 2-ring. The abundance of 5- and 4- ring PAHs in the selected sausage roll samples relative to 6-, 3- and 2-ring PAHs could be due to the baking process under high-temperature conditions above 220°C [25].

The PAH2, PAH4, and PAH8 levels are suitable indicators for total PAHs occurrence. Their ranged values ($\mu\text{g/g}$) are: PAH2 (1.53-3.36), PAH4 (3.83-18.0) and PAH8 (3.83-25.5). The LMW-PAHs -2, 3, 4-rings ranged (7.72-15.6 $\mu\text{g/g}$) while HMW - 5 and 6-rings ranged (1.29 $\mu\text{g/g}$ and 20.7 $\mu\text{g/g}$). The presence of high molecular weight PAHs in all the sausage roll samples may be due to combustion sources [26].

Table 2 shows the Benzo[a]pyrene equivalent factors for all brands of sausage rolls investigated in this study. The calculated Σ BaP_{eq} values in all samples analyzed ranged from 1.41 to 11.4 $\mu\text{g/g}$. The maximum concentration of Σ BaP_{eq} was observed in SB while the least concentration was observed in RT.

Table 3 showed the EDI of each of the 16 PAH compounds, Σ 16 PAHs, PAH2, PAH4, PAH8, and Σ BaP_{eq} for school children, youths, and adults. However, the daily dietary intake of PAHs for school children ranged from 2.38-4.32 $\mu\text{g/kg bw/day}$ (BaP), 3.50-7.68 $\mu\text{g/kg bw/day}$ (PAH2), 8.75-41.1 $\mu\text{g/kg bw/day}$ (PAH4), 8.75-58.3 $\mu\text{g/kg bw/day}$ (PAH8), 28.6-82.7 $\mu\text{g/kg bw/day}$ (Σ 16 PAHs) and 3.22-26.1 $\mu\text{g/kg bw/day}$ (Σ BaP_{eq}).

For youths ingesting the same amount of sausage roll per day, the dietary intake of BaP, PAH2, PAH4, PAH8, Σ 16 PAHs and Σ BaP_{eq} were in the range 1.28-2.33 $\mu\text{g/kg bw/day}$, 1.88-4.14 $\mu\text{g/kg bw/day}$, 4.71-22.2 $\mu\text{g/kg bw/day}$, 4.71-31.4 $\mu\text{g/kg bw/day}$, 15.4-44.6 $\mu\text{g/kg bw/day}$ and 1.74-14.0 $\mu\text{g/kg bw/day}$, respectively. Also the estimated daily intake of BaP, PAH2, PAH4, PAH8, Σ 16 PAHs and Σ BaP_{eq} for adults from sausage rolls ranged from 1.19-2.16 $\mu\text{g/kg bw/day}$, 1.75-3.84 $\mu\text{g/kg bw/day}$,

4.38-20.6 $\mu\text{g/kg bw/day}$, 4.38-29.1 $\mu\text{g/kg bw/day}$, 14.3-41.4 $\mu\text{g/kg bw/day}$ and 1.61-13.0 $\mu\text{g/kg bw/day}$, respectively.

In a study of PAHs in baked ready-to-eat food items in Nigeria conducted by Iwegbue [27], the estimated daily intakes of BaP, PAH2, PAH4 and PAH8 for an adult with an average body weight of 60 kg were in the range of 1.9 to 271.4 ng/kg bw/day . In general, the estimated dietary daily intakes of BaP and EFSA indicators for the occurrence of PAHs in foods like PAH2, PAH4, PAH8, in addition to Σ 16 PAHs and Σ BaP_{eq} from consumption of different brands of sausage rolls were much lower in adults relative to youths and school children. It should be known however that a school child that ingests the exact amount of sausage rolls as an adult for each day would of course be at higher risk of exposure to carcinogenic PAHs due to his/her small body weight and a lower rate of metabolism.

Margin of Exposure (MOE)

The MOE methodology was implemented to evaluate the risk of different indicators of occurrence and effects of PAHs in foods. The MOE is the relation between the benchmark dose lower limit (BMDL₁₀) and the estimated daily ingestion of the compound. The BMDL₁₀ values for BaP, PAH2, PAH4 and PAH8 are 0.07, 0.17, 0.34 and 0.49 mg/kg bw/day , respectively [6].

MOE values lower than 10,000 show serious health effects for consumers and require an immediate response. MOE values higher than 10,000 show no serious health concerns to consumers by the contaminants [26]. The MOE values of BaP, PAH2, PAH4, and PAH8 for school children, youths, and adults are displayed in Table 4. The calculated margin of exposure from the ingestion of all the sausage roll samples for all categories based on suitable indicators for PAHs occurrence was lower than 10,000, thus signifying severe health concerns. Consumers of these food products require abrupt attention because they are at greater risk of exposure to carcinogenic PAHs.

BaP Toxic Equivalent (TEQ)

The BaP toxic equivalent factor (BaP_{TEQ}) was utilized for the risk evaluation of PAHs in the environment [28]. The BaP toxic equivalent (BaP_{TEQ}) for each PAH was calculated as follows:

$$\text{BaP}_{\text{TEQ}} = \sum C_i \times \text{BaP}_{\text{TEF}} \quad (8)$$

Where,

Table 1: Concentrations ($\mu\text{g/g}$) of PAHs in sausage roll brands.

PAHs	Acronyms	BG	GA	SB	RT	YM
Naphthalene	Nap	0.16 \pm 0.02	ND	0.17 \pm 0.02	ND	ND
Acenaphthylene	Acy	0.19 \pm 0.00	0.29 \pm 0.00	0.13 \pm 0.01	ND	0.19 \pm 0.00
Acenaphthene	Ace	0.32 \pm 0.02	0.14 \pm 0.01	0.11 \pm 0.00	0.08 \pm 0.02	0.22 \pm 0.02
Fluorene	Flu	0.47 \pm 0.00	0.25 \pm 0.01	0.27 \pm 0.01	0.14 \pm 0.01	0.14 \pm 0.03
Phenanthrene	Phe	0.99 \pm 0.00	0.54 \pm 0.13	0.18 \pm 0.02	ND	0.62 \pm 0.01
Anthracene	Ant	0.73 \pm 0.00	0.42 \pm 0.14	0.28 \pm 0.02	ND	0.68 \pm 0.01
Fluoranthene	Flt	1.32 \pm 0.14	0.89 \pm 0.00	2.27 \pm 0.14	0.21 \pm 0.14	2.35 \pm 0.3
Pyrene	Pyr	5.19 \pm 0.14	2.36 \pm 0.13	7.28 \pm 0.02	8.19 \pm 0.14	0.24 \pm 0.3
Benz(a)anthracene	BaA	2.38 \pm 0.00	2.34 \pm 0.15	3.26 \pm 0.02	0.98 \pm 0.00	3.52 \pm 0.3
Chrysene	Chy	ND	0.49 \pm 0.00	1.60 \pm 0.13	1.56 \pm 0.13	1.47 \pm 0.03
Benzo(b)fluoranthene	BbF	4.49 \pm 0.03	2.11 \pm 0.13	4.26 \pm 0.14	ND	11.1 \pm 0.00
Benzo(k)fluoranthene	BkF	2.03 \pm 0.00	0.92 \pm 0.01	0.63 \pm 0.00	ND	1.60 \pm 0.01
Benzo(a)pyrene	BaP	1.85 \pm 0.00	1.04 \pm 0.02	1.18 \pm 0.01	1.29 \pm 0.00	1.89 \pm 0.00
Dibenz(a,h)anthracene	DahA	ND	6.61 \pm 0.00	9.18 \pm 0.14	ND	ND
Indeno(1,2,3-cd)pyrene	IcdP	ND	0.97 \pm 0.01	1.25 \pm 0.01	ND	ND
Benzo(g,h,i)perylene	BghiP	2.27 \pm 0.02	3.29 \pm 0.13	4.16 \pm 0.16	ND	1.82 \pm 0.13
Σ 16 PAHs		22.4 \pm 1.0	22.7 \pm 0.6	36.2 \pm 0.9	12.5 \pm 0.5	25.8 \pm 0.8
Σ 2-ring PAHs		0.16	0	0.17	0	0
Σ 3-ring PAHs		2.70	1.64	0.97	0.22	1.85
Σ 4-ring PAHs		8.89	6.08	14.4	10.9	7.58
Σ 5-ring PAHs		8.37	10.7	15.3	1.29	14.6
Σ 6-ring PAHs		2.27	4.26	5.41	0	1.82
PAH ₂		1.85	1.53	2.78	2.85	3.36
PAH ₄		8.72	5.98	10.3	3.83	18.0
PAH ₈		13.0	17.8	25.5	3.83	21.4
Σ LMW-PAHs		11.8	7.72	15.6	11.2	9.43
Σ HMW-PAHs		10.6	14.9	20.7	1.29	16.4

Table 2: BaP Equivalent Concentration (BaP_{eq}) (μg/g) in sausage roll.

PAHs	BG	GA	SB	RT	YM
NAP	0.16×10^{-4}	ND	0.17×10^{-3}	ND	ND
ACY	0.19×10^{-3}	0.29×10^{-3}	0.13×10^{-3}	ND	0.19×10^{-3}
ACE	0.32×10^{-3}	0.14×10^{-3}	0.11×10^{-3}	0.08×10^{-3}	0.22×10^{-3}
FLU	0.47×10^{-3}	0.25×10^{-3}	0.27×10^{-3}	0.14×10^{-3}	0.14×10^{-3}
PHE	0.99×10^{-3}	0.54×10^{-3}	0.18×10^{-3}	ND	0.62×10^{-3}
ANT	0.73×10^{-2}	0.42×10^{-2}	0.28×10^{-2}	ND	0.68×10^{-2}
FLT	1.32×10^{-2}	0.89×10^{-3}	2.27×10^{-3}	0.21×10^{-3}	2.35×10^{-3}
PYR	5.19×10^{-3}	2.36×10^{-3}	7.28×10^{-3}	8.19×10^{-3}	0.24×10^{-3}
BaA	2.38×10^{-1}	2.34×10^{-1}	3.26×10^{-1}	9.08×10^{-2}	3.52×10^{-1}
CHY	ND	4.9×10^{-2}	1.6×10^{-2}	1.56×10^{-2}	1.47×10^{-2}
BbF	4.49×10^{-1}	2.11×10^{-1}	4.26×10^{-1}	ND	1.11
BkF	2.03×10^{-1}	9.2×10^{-2}	6.3×10^{-2}	ND	1.60×10^{-1}
BaP	1.85	1.04	1.18	1.29	1.89
DahA	ND	6.61	9.18	ND	ND
IcdP	ND	9.7×10^{-2}	1.25×10^{-1}	ND	ND
BghiP	2.27×10^{-2}	3.29×10^{-2}	4.16×10^{-2}	ND	1.82×10^{-2}
Σ BaP _{eq}	2.76	8.33	11.4	1.41	3.56

BaP_{TEF} = Cancer Potency relative to BaP

C_i = Concentration of individual PAH (μg/g)

The BaP carcinogenic equivalency factors (BaP_{TEF}) of the thirteen carcinogenic PAHs used were ANT=0.01, BaA = 0.1, BaP = 1, BbF = 0.1, BkF = 0.01, CHY = 0.001, DahA = 1, FLT=0.001, FLU=0.001, IcdP = 0.1, NAP=0.001, PHE=0.001 and PYR=0.001 [29]. The BaP_{TEQ} for sausage roll brands are presented in Table 5. The Σ BaP_{TEQ} values ranged from 1.40 to 11.3 μg/g. The major contributors to the Σ BaP_{TEQ} value are BaA, BbF, BaP and DahA. GA and SB had higher carcinogenic potency factor than any other samples examined.

BaP Mutagenic Equivalent (MEQ)

The BaP Mutagenic Equivalent (MEQ) which deals with mutagenic activities may indirectly be related to cancer [30-31] and could implicitly be associated with

non-cancer effects like pulmonary diseases, birth defects, impotency, low IQ, etc. [32-33].

The BaP mutagenic potency factors (BaP_{MEF}) were BaP =1, BaA = 0.082, BbF = 0.25, BkF = 0.11, CHY = 0.017, DahA = 0.29, IcdP = 0.31 and BghiP = 0.19 [34]. The overall mutagenic potency factor for these sausage roll brands ranged from 1.40 to 6.45 μg/g with substantial contributions from BaP, BbF, and DahA. SB and YM had the higher mutagenic potency factors than any brands investigated (Table 6).

Incremental Lifetime Cancer Risk (ILCR) for PAHs

The USEPA suggested that a one-in-a-million risk of additional human cancer over a 70-year lifetime (ILCR=10⁻⁶) is the level of risk deliberated to be acceptable or insignificant [35]. Supplementary lifetime cancer risk of one in ten thousand or greater (ILCR=10⁻⁴)

Table 3: Estimated Dietary Daily Intakes ($\mu\text{g}/\text{kg bw}/\text{day}$) for School Children (35 kg), Youths (65 kg) and Adults (70 kg) from Ingestion of 80 g of Sausage Roll Samples.

	School Child					Youth					Adult				
	BG	GA	SB	RT	YM	BG	GA	SB	RT	YM	BG	GA	SB	RT	YM
NAP	0.366	0.000	0.389	0.000	0.000	0.197	0.000	0.209	0.000	0.000	0.183	0.000	0.194	0.000	0.000
ACY	0.434	0.663	0.297	0.000	0.434	0.234	0.357	0.160	0.000	0.234	0.217	0.331	0.149	0.000	0.217
ACE	0.731	0.320	0.251	0.183	0.503	0.394	0.172	0.135	0.099	0.271	0.366	0.160	0.126	0.091	0.251
FLU	1.074	0.571	0.617	0.320	0.320	0.578	0.308	0.332	0.172	0.172	0.537	0.286	0.309	0.160	0.160
PHE	2.263	1.234	0.411	0.000	1.417	1.218	0.665	0.222	0.000	0.763	1.131	0.617	0.206	0.000	0.709
ANT	1.669	0.960	0.64	0.000	1.554	0.898	0.517	0.345	0.000	0.837	8.343	0.480	0.320	0.000	0.777
FLT	3.017	2.034	5.189	0.480	5.371	1.625	1.095	2.794	0.258	2.892	1.509	1.017	2.594	0.240	2.686
PYR	11.862	5.394	16.640	18.720	0.549	6.388	2.905	8.960	10.080	0.295	5.931	2.697	8.320	9.360	0.274
BaA	5.440	5.349	7.451	2.240	8.046	2.929	2.880	4.012	1.206	4.332	2.720	2.674	3.726	1.120	4.023
CHY	0.000	1.120	3.657	3.566	3.360	0.000	0.603	1.969	1.920	1.809	0.000	0.560	1.829	1.783	1.680
BbF	10.263	4.823	9.737	0.000	25.371	5.526	2.597	5.243	0.000	13.661	5.131	2.411	4.869	0.000	12.686
BkF	4.640	2.103	1.440	0.000	3.657	2.498	1.132	0.775	0.000	1.969	2.320	1.051	0.720	0.000	1.829
BaP	4.229	2.377	2.697	2.949	4.320	2.277	1.280	1.452	1.588	2.326	2.114	1.189	1.349	1.474	2.160
DahA	0.000	15.109	20.983	0.000	0.000	0.000	8.135	11.298	0.000	0.000	0.000	7.554	10.491	0.000	0.000
IcdP	0.000	2.217	2.857	0.000	0.000	0.000	1.194	1.538	0.000	0.000	0.000	1.109	1.429	0.000	0.000
BghiP	5.189	7.520	9.509	0.000	4.160	2.794	4.049	5.120	0.000	2.240	2.594	3.760	4.754	0.000	2.080
Σ 16 PAHs	51.200	51.886	82.743	28.571	58.971	27.569	27.938	44.554	15.385	31.754	25.600	25.943	41.371	14.286	29.486
PAH2	4.229	3.497	6.354	6.514	7.680	2.277	1.883	3.422	3.508	4.135	2.114	1.749	3.177	3.257	3.840
PAH4	19.931	13.669	23.543	8.754	41.143	10.732	7.360	12.677	4.714	22.154	9.966	6.834	11.771	4.377	20.571
PAH8	29.714	40.686	58.286	8.754	48.914	16.000	21.908	31.385	4.714	26.338	14.857	20.343	29.143	4.377	24.457
Σ BaP _{eq}	6.309	19.040	26.057	3.223	8.137	3.397	10.252	14.031	1.735	4.382	3.154	9.520	13.029	1.611	4.069

Table 4: Margin of Exposure (MOE) from Ingestion of 80 g of sausage roll for School Children (35 kg), Youths (65 kg) and Adults (70 kg).

Sausage Sample	Margin of Exposure			
	BaP	PAH2	PAH4	PAH8
SCHOOL CHILD				
BG	16.5	40.2	17.1	16.5
GA	29.4	48.6	24.8	12.0
SB	25.9	26.8	14.5	8.40
RT	23.7	26.1	38.9	56.0
YM	16.2	22.1	8.27	10.0
YOUTH				
BG	30.7	74.6	31.8	30.6
GA	54.7	90.4	46.2	22.4
SB	48.3	49.7	26.8	15.6
RT	25.2	48.4	72.2	104
YM	30.0	41.1	15.3	18.6
ADULT				
BG	33.2	80.6	34.1	32.9
GA	58.8	97.1	49.8	24.1
SB	51.9	53.5	28.8	16.8
RT	47.6	52.1	77.6	112
YM	32.4	44.3	16.5	20.0

Table 5: Calculated BaP Toxic Equivalent (BaPTEQ) ($\mu\text{g/g}$) via the Ingestion of 80 g of Sausage roll for each day.

	BG	GA	SB	RT	YM
NAP	1.6×10^{-4}	0	1.7×10^{-4}	0	0
FLU	4.7×10^{-4}	2.5×10^{-4}	2.7×10^{-4}	1.4×10^{-4}	1.4×10^{-4}
PHE	9.9×10^{-4}	5.4×10^{-4}	1.8×10^{-4}	0	6.2×10^{-4}
ANT	7.3×10^{-3}	4.2×10^{-3}	2.8×10^{-3}	0	6.8×10^{-3}
FLT	1.3×10^{-3}	8.9×10^{-4}	2.3×10^{-3}	2.1×10^{-4}	2.4×10^{-3}
PYR	5.2×10^{-3}	2.36×10^{-3}	7.3×10^{-3}	8.2×10^{-3}	2.4×10^{-4}
BaA	2.4×10^{-1}	2.3×10^{-1}	3.3×10^{-1}	9.8×10^{-2}	3.5×10^{-1}
CHY	0	4.9×10^{-4}	1.6×10^{-3}	1.6×10^{-3}	1.5×10^{-3}
BbF	4.5×10^{-1}	2.1×10^{-1}	4.26×10^{-1}	0	1.11
BkF	2.03×10^{-2}	9.2×10^{-3}	6.3×10^{-3}	0	1.6×10^{-2}
BaP	1.85	1.04	1.18	1.29	1.89
DahA	0	6.61	9.18	0	0
IcdP	0	9.7×10^{-2}	1.25×10^{-1}	0	0
$\Sigma \text{BaP}_{\text{TEQ}}$	2.57	8.21	11.3	1.40	3.38

Table 6: Calculated BaP Mutagenic Equivalent (BaPMEQ) ($\mu\text{g/g}$) via the Ingestion of 80 g of Sausage roll for each day.

	BG	GA	SB	RT	YM
BaA	1.95×10^{-1}	1.92×10^{-1}	2.67×10^{-3}	8.04×10^{-2}	0.289
CHY	0	0.00833	0.0272	0.0265	0.0250
BbF	1.12	0.528	1.07	0	2.78
BkF	0.223	0.101	0.0693	0	0.176
BaP	1.85	1.04	1.18	1.29	1.89
DahA	0	1.92	2.66	0	0
IcdP	0	0.301	0.388	0	0
BghiP	0.431	0.625	0.790	0	0.346
$\Sigma \text{BaP}_{\text{MEQ}}$	3.82	4.72	6.45	1.40	5.51

is deemed critical and there is great urgency for giving consideration to such health challenges.

The cumulative probability distributions of the calculated ILCR for school children, youths, and adults from the consumption of 80 g sausage roll are displayed in Table 7. The Σ ILCR for school children was in the range of 7.91×10^{-6} to 1.08×10^{-5} while youths and adults had values in the range of 9.57×10^{-7} - 1.46×10^{-6} and 8.25×10^{-7} - 2.70×10^{-6} , respectively. These values are higher than the acceptable risk level (1.0×10^{-6}) and lower than the priority risk level (1.0×10^{-4}).

Trace Metal

Mean concentrations of trace metals in sausage roll samples

Table 8 presents the concentration of trace metals in selected brands of sausage rolls. Zn ranged from 1.01 mg/kg in SB to 71 mg/kg in GA. The concentration of Zn obtained in these sausage roll samples analyzed was below 99.4 mg/kg maximum permissible level provided by FAO/WHO [36] for cereal-based food products. Zinc is an essential metal that helps to speed up the healing process after injury and it is required for normal growth and development during pregnancy. Zn level in sausage has been reported by Demirezen and Uruc [37] to be as low as 0.6 mg/kg. Pb ranged from 2.34 mg/kg in YM to 13.0 mg/kg in GA. The concentrations of Pb in all the products were above the maximum permissible level of 0.3 mg/kg and 0.05 mg/kg set by FAO/WHO [36] and EC [38], respectively. Cu ranged from not detected in BG to 8.12 mg/kg in GA. The concentration of Cu in all the brands was below the permissible level stipulated by FAO/WHO [36] and EC [38].

Cu is a co-factor to many enzymes and plays a key role in the absorption, utilization, and transport of Fe in the human body to prevent Fe deficiency. The values of Cu reported in this study are quite higher than the values obtained by Gopalani *et al.* [39] who reported ND-4.70 mg/kg Cu in biscuits from Nagpur, India. The concentration of Cr in a sausage roll sample was only detected in GA with a value of 16.4 mg/kg. Iwegbue *et al.* [40] reported the mean level of Cr in sausage rolls as 15.1 mg/kg. Cr level in GA surpassed the tolerable limit set by FAO/WHO [36] for cereal-based food products. Cd was not detected in all the sausage roll samples. Thus, Cd meets the FAO/WHO and EC requirements (safety limits of 0.2 mg/kg and 0.04 mg/kg, respectively), since it was not detected in all the samples.

ND = Not detected

FAO/WHO = Food and Agricultural Organization/World Health Organization [36] safe limit for cereal-based food products

EC = European Commission [38] safe limit for processed cereal-based foods

Estimated Daily Intake (EDI) of trace metals

The estimated daily intake of selected metals from ingestion of 80 g of various brands of sausage roll per day for school children, youths, and adults is presented in Table 9.

The recommended daily intake by the Institute of Medicine [41], National Research Council of Canada [42], WHO Provisional Maximal Tolerable Daily Intake (PMTDI) [43], and Joint Expert Committee on Food Additives (JECFA) PMTDI for Zn is set at 40,000 $\mu\text{g/day}$ for adults within the ages of 19 - 70, 12,000 $\mu\text{g/day}$, 0.3 mg/kg/bw/day and 1000 $\mu\text{g/kg/bw/day}$ respectively. However, for this

Table 7: Estimated Incremental Lifetime Cancer Risk (ILCR) from the Ingestion of 80 g of Sausage roll for School Children, Youths and Adults.

	BG	GA	SB	RT	YM
School Child					
NAP	1.24×10^{-9}	0.000	1.32×10^{-9}	0.000	0.000
BaA	1.13×10^{-7}	1.12×10^{-7}	1.55×10^{-7}	4.67×10^{-8}	1.68×10^{-7}
CHY	0.000	2.34×10^{-10}	7.63×10^{-10}	7.44×10^{-10}	7.01×10^{-10}
BbF	2.14×10^{-7}	1.01×10^{-7}	2.03×10^{-7}	0.000	5.29×10^{-7}
BkF	9.68×10^{-9}	4.39×10^{-9}	3.00×10^{-9}	0.000	7.63×10^{-9}
BaP	8.82×10^{-7}	4.96×10^{-7}	5.63×10^{-7}	6.15×10^{-7}	9.01×10^{-7}
DahA	0.000	3.15×10^{-6}	4.38×10^{-6}	0.000	0.000
IcdP	0.000	4.62×10^{-8}	5.96×10^{-8}	0.000	0.00
Σ BaP _{eq}	1.32×10^{-6}	3.97×10^{-6}	5.43×10^{-6}	6.72×10^{-7}	1.70×10^{-6}
Σ ILCR	2.53×10^{-6}	7.91×10^{-6}	1.08×10^{-5}	1.33×10^{-6}	3.31×10^{-6}
Youth					
NAP	3.61×10^{-10}	0.000	3.83×10^{-10}	0.000	0.000
BaA	3.29×10^{-8}	3.23×10^{-8}	4.51×10^{-8}	1.35×10^{-8}	4.87×10^{-8}
CHY	0.000	6.77×10^{-11}	2.21×10^{-10}	2.16×10^{-10}	2.81×10^{-10}
BbF	6.21×10^{-8}	2.92×10^{-8}	5.89×10^{-8}	0.000	1.53×10^{-7}
BkF	2.81×10^{-9}	1.27×10^{-9}	8.70×10^{-10}	0.000	2.21×10^{-9}
BaP	2.56×10^{-7}	1.44×10^{-7}	1.63×10^{-7}	1.78×10^{-7}	2.61×10^{-7}
DahA	0.000	9.14×10^{-7}	1.27×10^{-6}	0.000	0.000
IcdP	0.000	1.34×10^{-8}	1.73×10^{-8}	0.000	0.000
Σ BaP _{eq}	3.82×10^{-7}	1.15×10^{-6}	1.58×10^{-6}	1.95×10^{-7}	4.92×10^{-7}
Σ ILCR	7.36×10^{-7}	1.46×10^{-6}	3.14×10^{-6}	3.87×10^{-7}	9.57×10^{-7}
Adult					
NAP	3.11×10^{-10}	0.000	3.30×10^{-10}	0.000	0.000
BaA	2.84×10^{-8}	2.79×10^{-8}	3.89×10^{-8}	1.17×10^{-8}	4.20×10^{-8}
CHY	0.000	5.84×10^{-11}	1.91×10^{-10}	1.86×10^{-10}	1.75×10^{-10}
BbF	5.35×10^{-8}	2.51×10^{-8}	5.08×10^{-8}	0.000	1.32×10^{-7}
BkF	2.42×10^{-9}	1.10×10^{-9}	7.51×10^{-10}	0.000	1.91×10^{-9}
BaP	2.20×10^{-7}	1.24×10^{-7}	1.41×10^{-7}	1.54×10^{-7}	2.25×10^{-7}
DahA	0.000	7.88×10^{-7}	1.09×10^{-6}	0.000	0.000
IcdP	0.000	1.16×10^{-8}	1.49×10^{-8}	0.000	0.000
Σ BaP _{eq}	3.29×10^{-7}	9.93×10^{-7}	1.36×10^{-6}	1.68×10^{-7}	4.24×10^{-7}
Σ ILCR	6.34×10^{-7}	1.97×10^{-6}	2.70×10^{-6}	3.34×10^{-7}	8.25×10^{-7}

Table 8: Trace metals in sausage roll (mg/kg).

Sausage roll Sample	Trace Metals (mg/kg)				
	Zn	Pb	Cu	Cr	Cd
BG	7.30±0.3	6.37±1.7	ND	ND	ND
GA	71.0±1.4	13.0±1.5	8.12±1.4	16.4±2.8	ND
RT	2.20±1.4	12.6±2.8	5.52±0.8	ND	ND
SB	1.01±1.4	4.80±0.0	4.80±0.0	ND	ND
YM	1.78±1.5	2.34±3.4	2.34±3.4	ND	ND
FAO/WHO Safe limit	99.4	0.3	73.3	2.3	0.2
EC Safe limit	150	0.05	10	-	0.04

the present research, the estimated dietary intake of Zn was in the ranges of 2.31 – 162 µg/kg bw/day for a schoolchild, 1.24 – 87.4 µg/kg bw/day for youth, and 1.15 – 81.1 µg/kg bw/day for an adult. These EDI of Zn values for all categories were far lower than the recommended daily intake.

The estimated daily intake of Pb in this research was between 5.35 and 29.7 µg/kg bw/day for school children scenario, 2.88 and 16.0 µg/kg bw/day for youth scenario, 2.67 and 14.9 µg/kg bw/day for an adult scenario. The values in this study for all categories were higher than JECFA Provisional Tolerable Weekly Intake (PTWI) of Pb recommended at 25 µg/kg/bw for infants. However, this value has been retracted in 2011 by the joint FAO/WHO committee since it was not counted to be safe for human health [43]. Only YM has intakes lower than the JECFA PTWI for youth and adult scenarios.

The estimated daily exposure to Cu from these sausage roll products in this study is between 0.00-18.6 µg/kg bw/day, 0.00-9.99 µg/kg bw/day, and 0.00-9.28 µg/kg bw/day for school children, youths, and adults scenario, respectively. The Expert Group on Vitamins and Minerals recommended a safe maximum limit of Cu to be 160 µg/kg bw/day [44]. The values in this study are lower than the recommended value.

The EDIs for Chromium in this research spanned from 0.00 to 37.5 µg/kg bw/day for a schoolchild, while that of a youth and an adult that ingested the exact amount of sausage rolls for each day varied between 0.00 to 20.2 µg/kg bw/day and 0.00 to 18.7 µg/kg bw/day respectively. Joint FAO/WHO Expert Committee of Food Additives (JECFA) fixed a maximum permissible daily intake for Chromium as 200 µg/day [43] and the Expert Group on Vitamins and Minerals (EVM) safe maximum limit for Chromium is 150 µg/kg bw/day [44]. The EDIs for Chromium for the case

of a school child make up highly 18.8% of the EVM guidance level and 25% for JECFA recommended limit, 10.1%, and 9.35% of the JECFA limit for youth and adults respectively. The estimated dietary daily intake of Cr constituted 13.5% and 12.4% of the EVM level for youth and adults, respectively.

Target Hazard Quotient (THQ)

In this present work, Σ THQ was calculated by adding the THQ value of each metal. However, the higher the Σ THQ value, the greater the level of concern.

The estimated target hazard quotients of selected trace metals through the consumption of 80 g of sausage rolls for school children, youths, and adults in Nigeria are displayed in Table 10. Zn, Cu, and Cd in all samples for all scenarios had THQ < 1, while Cr in all samples apart from GA had THQ < 1 for all scenarios signifying no serious health concern. The THQ of Pb was > 1 for all scenarios apart from YM for youths and adults, thus indicating the potential for serious health concerns. The values of THQ among the different levels were in the order: adults < youths < school children. The individual THQ values for trace metals for all scenarios follow the order: Pb > Cr > Cu > Zn > Cd. The Σ THQ values of analyzed metals for sausage roll brands for all scenarios ranged from 0.74 to 20.9. The lead had the greatest effects on the Σ THQ values than other metals while Cd had no feasible effects.

Incremental Lifetime Carcinogenic Risk (ILCR) of Heavy Metals

The chance of cancer risks in the examined sausage roll samples via the ingestion of carcinogenic trace metals was calculated by means of the Incremental Lifetime Cancer Risk (ILCR). The carcinogenic risk of both Pb and Cr were calculated. The equation that was applied for estimation of the cancer risk is as follows [45]:

Table 9: Estimated Daily Intake of Metals ($\mu\text{g}/\text{kg bw}/\text{day}$) based on Consumption of 80 g of Sausage roll for School Children (35 kg), Youths (65 kg) and Adults (70 kg).

Sausage roll Sample	Zn	Pb	Cu	Cr	Cd
School Child					
BG	16.7	14.6	0.00	0.00	0.00
GA	162	29.7	18.6	37.5	0.00
RT	5.03	28.8	12.6	0.00	0.00
SB	2.31	11.0	11.0	0.00	0.00
YM	4.07	5.35	5.35	0.00	0.00
Youth					
BG	8.98	7.84	0.00	0.00	0.00
GA	87.4	16.0	9.99	20.2	0.00
RT	2.71	15.5	6.79	0.00	0.00
SB	1.24	5.91	5.91	0.00	0.00
YM	2.19	2.88	2.88	0.00	0.00
Adult					
BG	8.34	7.28	0.00	0.00	0.00
GA	81.1	14.9	9.28	18.7	0.00
RT	2.51	14.4	6.31	0.00	0.00
SB	1.15	5.49	5.49	0.00	0.00
YM	2.03	2.67	2.67	0.00	0.00

Table 10: Estimated Target Hazard Quotients (THQ) of Heavy Metals from the Consumption of 80 g of Sausage rolls for School Children, Youths and Adults.

Sausage roll Sample	Zn	Pb	Cu	Cr	Cd	Σ THQ
School Child						
BG	0.056	3.65	0.000	0.00	0.00	3.71
GA	0.540	7.43	0.465	12.5	0.00	20.9
RT	0.017	7.20	0.315	0.00	0.00	7.53
SB	0.008	2.75	0.275	0.00	0.00	3.03
YM	0.014	1.34	0.134	0.00	0.00	1.49
Youth						
BG	0.030	1.96	0.000	0.00	0.00	1.99
GA	0.291	4.00	0.250	6.73	0.00	11.3
RT	0.009	3.88	0.170	0.00	0.00	4.06
SB	0.004	1.48	0.148	0.00	0.00	1.63
YM	0.007	0.72	0.072	0.00	0.00	0.80
Adult						
BG	0.028	1.82	0.000	0.00	0.00	1.85
GA	0.270	3.73	0.232	6.23	0.00	10.5
RT	0.008	3.60	0.158	0.00	0.00	3.77
SB	0.004	1.37	0.137	0.00	0.00	1.51
YM	0.007	0.67	0.067	0.00	0.00	0.74

Table 11: Carcinogenic Risk of Trace Metals in Selected Sausage roll brands for School Children, Youths and Adults .

Sausage roll Sample	Zn	Pb	Cu	Cr	Cd	Σ ILCR
School Child						
BG	-	1.24×10^{-4}	-	-	-	1.24×10^{-4}
GA	-	2.52×10^{-4}	-	1.88×10^{-2}	-	1.91×10^{-2}
RT	-	2.45×10^{-4}	-	-	-	2.45×10^{-4}
SB	-	9.35×10^{-5}	-	-	-	9.35×10^{-5}
YM	-	4.55×10^{-5}	-	-	-	4.55×10^{-5}
Youth						
BG	-	6.66×10^{-5}	-	-	-	6.66×10^{-5}
GA	-	1.36×10^{-4}	-	1.01×10^{-2}	-	1.02×10^{-2}
RT	-	1.32×10^{-4}	-	-	-	1.32×10^{-4}
SB	-	5.02×10^{-5}	-	-	-	5.02×10^{-5}
YM	-	2.45×10^{-5}	-	-	-	2.45×10^{-5}
Adult						
BG	-	6.19×10^{-5}	-	-	-	6.19×10^{-5}
GA	-	1.27×10^{-4}	-	9.35×10^{-3}	-	9.48×10^{-3}
RT	-	1.22×10^{-4}	-	-	-	1.22×10^{-4}
SB	-	4.67×10^{-5}	-	-	-	4.67×10^{-5}
YM	-	2.27×10^{-5}	-	-	-	2.27×10^{-5}

$$ILCR = CSF \times EDI \quad (9)$$

Where, CSF is the carcinogenic slope factor for Pb and Cr were 0.0085 mg/kg/day and 0.5 mg/kg/day, respectively and EDI is the estimated daily intake of individual metals ($\mu\text{g}/\text{kg}/\text{bw}/\text{day}$). Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000). Moderate risk level ($ILCR > 10^{-3}$) and ($ILCR > 10^{-2}$) that is above 1 in 1,000 and 1 in 100 respectively are considered unacceptable and the health safety of the public is of concern. The collective cancer risk resulting from exposure to numerous carcinogenic heavy metals because of ingestion of a specific brand of sausage roll was adopted to be the addition of each trace metal with an increase in risks [45].

The computed values for ILCR and summation of Incremental Lifetime Cancer Risk (Σ ILCR) for both Pb and Cr due to the exposure from the ingestion of 80 g of sausage roll are presented in Table 11. In this present study, the values of ILCR for Cr breached the acceptable

range of ($> 10^{-4}$) in all studied samples of sausage rolls with a range of 9.35×10^{-3} to 1.01×10^{-2} whereas the ILCR values for Pb were between 10^{-6} and 10^{-4} having a range between 2.27×10^{-5} and 2.45×10^{-4} . However, the values for Pb for all categories and in all samples are considered acceptable. Therefore, the consumption of sausage rolls is less risky in this study and less vulnerable to cancer risk. Moreover, Cr is less carcinogenic and the values obtained in this study for the consumption of sausage rolls are of less concern to the general population.

CONCLUSIONS

The concentration of the Σ 16 PAHs in five brands of a sausage roll in the Nigerian markets showed the prevalence of 5-ring PAHs and 4-ring PAHs over the 2-ring, 3-ring, and 6-ring PAHs. In addition, the high molecular mass 4- to 6-ringed PAHs also showed dominance over the low molecular mass 2- and 3- ringed PAHs in the majority of these samples. However, all the brands had BaP concentrations greater than $1 \mu\text{g}/\text{kg}$, which

is the maximum permissible limit stipulated for processed cereal-based food products by the European Commission. The MOE values were lower than 10,000 in all the brands based on BaP, PAH2, PAH4, and PAH8 for all scenarios studied. It has been stated by EFSA that MOE values less than 10,000 require immediate attention to consumers' health. The concentrations of trace metals in various sausage roll brands were quite low except Pb and Cr which recorded higher concentrations than their permissible limits in cereal-based food products. Cu was only present in GA and Cd was not detected in all the sausage roll brands. All other metals present in the sausage roll samples were at concentrations below the permissible limit. The Estimated Daily Intake values showed that the intakes of metals from the ingestion of these brands of sausage rolls were within their provisional tolerable daily intake limits for the toxic metals and recommended daily intake values for the essential metals. The intakes of beneficial metals like Zn from the ingestion of these products were low and contribute quite significantly to the dietary requirement of Zn. Consequentially, excessive ingestion of these products could result in exposure to high concentrations of metals; therefore parents should guide their wards by controlling their consumption rate of these food products. The concentration of contaminants found in these brands of sausage proved that there is an urgent need for limits to be stipulated for PAHs and trace metals in foods in Nigeria, and proficient observations of PAHs in food for potential health risks should be taken into action.

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REFERENCES

- [1] Plaza-Bolanos P., Frenich A.G., Vidal J.L.M., Polycyclic Aromatic Hydrocarbons in Food and Beverages, *Analytical Methods and Trends. J. Chromatography A*, **1217(41)**: 6303-6326 (2010).
- [2] Viegas O, Novo P., Pinto E., Pincho O., Ferreira IMPLV.O., Effect of Charcoal Types and Grilling Conditions on Formation of Heterocyclic Aromatic Amines (HAs) and Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Muscles Foods, *Food and Chemical Toxicology*, **50(6)**: 2128-2134 (2012).
- [3] Onwukeme V.I., Obijiofor O.C., Asomugha R.N., Okafor F.A., Impact of Cooking Methods on the Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Chicken Meat, *IOSR J Environ. Sci., Toxicol. and Food Technol.*, **9**: 21-7 (2015).
- [4] Bock S., Diagnosis and Treatment of Heavy Metals Toxicity. *Int. J. Integrative Medicines*, **1(6)**: 7-12 (1999).
- [5] Garcia-Falcon M.S., Perez-Lomela C., Simal-Gandara J., Strategies for the Extraction of Free and Bound Polycyclic Aromatic Hydrocarbons in Run-off Water Rich in Organic Matter, *Analytica Chimica Acta*, **508(2)**: 177-183 (2004).
- [6] European Food Safety Authority (EFSA), Polycyclic Aromatic Hydrocarbons in Food: Scientific Opinion of the Panel on Contaminants in the Food Chain, *The EFSA Journal*, **724**: 1-114 (2008).
- [7] Ciecierska M., Obiedziński M.W., Polycyclic Aromatic Hydrocarbons in the Bakery Chain, *Food Chem.*, **141(1)**: 1-9 (2013).
- [8] Ramesh A., Walker S.A., Hood D.B., Guillén M.D., Schneider K., Weyand E.H., Bioavailability and Risk Assessment of Orally Ingested Polycyclic Aromatic Hydrocarbons, *Int. J. Toxicol.*, **23(5)**: 301-333 (2004)
- [9] Alomirah H., Al-Zenki S., Al-Hooti S., Zaghoul S., Sawaya W., Ahmed N., Kannan K., Concentrations and Dietary Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) from Grilled and Smoked Foods, *Food Control*, **22(12)**: 2028-2035 (2011)
- [10] USEPA (United States, Environmental Protection Agency), Regional Screening Levels (RSLs) – Users Guide. U.S. Environmental Protection Agency. <https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide-june-2017> (2017).
- [11] IPCS (International Programme on Chemical Safety), Polycyclic Aromatic Hydrocarbons, Selected Non-Heterocyclic. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc202.htm>. (2010)
- [12] Xia Z., Duan X., Qiu W, Liu D., Wang B., Tao S., Jiang Q., Lu B., Song Y., Hu X., Health Risk Assessment on Dietary Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) in Taiyuan, China. *Science of the Total Environment*, **408(22)**: 5331-5337 (2010)

- [13] Onianwa P.C., Adetola I.G., Iwegbue C.M.A., Ojo M.F., Tella O.O., [Trace Heavy Metals Composition of some Nigerian Beverages and Food Drinks](#), *Food Chemistry*, **66**(3): 275-279 (1999).
- [14] Gopalani M., Shahare M., Ramteke D.S., Wate S.R., [Heavy Metal Content of Potato Chips and Biscuits from Nagpur City, India](#), *Bulletin of Environmental Contamination and Toxicology*, **79**(4): 384-387 (2007).
- [15] Iwegbue C.M.A., [Composition and Daily Intakes of Some Trace Metals from Canned Beers in Nigeria](#), *Journal of the Institute of Brewing*, **116**(3): 312-315 (2010).
- [16] Suppin D., Zahlbruckner R., Krapfenbauer-Cermak C.H., Hassan-Hauser C.H., Smulders F.J.M., [Mercury, Lead and Cadmium Content of Fresh and Canned Fish Collected from Austrian Retail Operations](#), *Nutrition*, **29**(11): 456-460 (2005).
- [17] Chisolm J.J., [Chronic Lead Intoxication in Children](#). *Developmental Medicine & Child Neurology*, **7**(5): 529-536 (1965).
- [18] Sodhi G.S., "Fundamental Concepts of Environmental Chemistry", 2nd ed. Narosa Publishing House, New Delhi, 332-351(2006)
- [19] Nisbet I.C., LaGoy P.K., [Toxic Equivalency Factors \(TEFs\) for Polycyclic Aromatic Hydrocarbons \(PAHs\)](#), *Regulatory Toxicology and Pharmacology*, **16**(3): 290-300 (1992).
- [20] Udowelle N.A., Igweze Z.N., Asomugha R.N., Orisakwe O.E., [Health Risk Assessment and Dietary Exposure of Polycyclic Aromatic Hydrocarbons \(PAHs\), Lead and Cadmium from Bread consumed in Nigeria](#), *Roczniki Państwowego Zakładu Higieny*, **68**(3):269-280 (2017).
- [21] KDHE (Kansas Department of Health and Environment), [Risk-based Standards for Kansas. RSK Manual, 5th version](#). KDHE, Topeka, KS, USA. Available at: www.kdheks.gov/remedial/download/RSK_Manual_10.pdf.(2010).
- [22] Iwegbue C.M., Basse F.I., Tesi G.O., Overah L.C., Onyeloni, S.O., Martincigh B.S., [Concentrations and Health Risk Assessment of Metals in Chewing Gums, Peppermints and Sweets in Nigeria](#), *Journal of Food Measurement and Characterization*, **9**(2): 160-174 (2015).
- [23] Aigberua A.O., Izah S.C., Isaac U.I., [Level and Health Risk Assessment of Heavy Metals in Selected Seasonings and Culinary Condiments Used in Nigeria](#), *Biological Evidence*, **8**(2): 6-20 (2018).
- [24] Naughton D.P., Petroczi A., [Heavy Metal ions in Wines: Meta-Analysis of Target Hazard Quotient Reveals Health Risk](#), *Chemistry Central Journal*, **2**: 22 (2008)
- [25] Houessou J.K., Maloug S., Leveque A.S., Delteil C., Heyd B., Camel V., [Effect of Roasting Conditions on the Polycyclic Aromatic Hydrocarbon Content in Ground Arabica Coffee and Coffee Brew](#), *Journal of Agricultural and Food Chemistry*, **55**(23): 9719-9726 (2007)
- [26] ATSDR (Agency for Toxic Substances and Disease Registry), "Toxicological Profile for Polycyclic Aromatic Hydrocarbons". US Department of Health and Human Services. US Government Printing Office, 639-298 {1995}
- [27] Iwegbue C.M., [Concentrations and Hazards of Polycyclic Aromatic Hydrocarbons in Hawked Baked Ready-to-Eat Foods in Nigeria](#), *Acta Alimentaria*, **45**(2): 175-181 (2016)
- [28] Durant J.L., Lafleur A.L., Busby W.F., Donhoffner L.L., Penman B.W., Crespi C.L., [Mutagenicity of C24 H14 PAH in Human Cells Expressing CYP1A1](#). *Mutation Research/Genetic, Toxicology and Environmental Mutagenesis*, **446**(1):1-14 (1999)
- [29] Bansal V., Kim K.H., [Review of PAH Contamination in Food Products and their Health Hazards](#), *Environ Intern*, **84**: 26-38 (2015)
- [30] Zeiger E., [Identification of Rodent Carcinogens and Non-Carcinogens using Genetic Toxicity Tests: Premises, Promises, and Performance](#), *Reg Toxicol and Pharmacol*, **28**: 85-95 (1998).
- [31] Zeiger E., [Mutagens that are not Carcinogens: Faulty Theory or Faulty Tests? Mutation Research/Genetic, Toxicology and Environmental Mutagenesis](#), **492**: 29-38 (2001)
- [32] DeMarini D.M., Brooks L.R., Warren S.H., Kobayashib T., Gilmour M.I., Singh P., [Bioassay-Directed Fractionation and Salmonella Mutagenicity of Automobile and Forklift Diesel Exhaust Particles](#), *Environ Health Perspect*, **112**(8): 814 (2004).

- [33] Seagrave J., McDonald J.D., Gigliotti A.P., Nikula K.J., Seilkop S.K., Gurevich M., Mauderly J.L., *Mutagenicity and in vivo Toxicity of Combined Particulate and Semi-volatile Organic Fractions of Gasoline and Diesel Engine Emissions*, *Toxicol. Sci.*, **70(2)**: 212-226 (2002).
- [34] Durant J.L., Busby Jr W.F., Lafleur A.L., Penman B.W., Crespi C.L., *Human Cell Mutagenicity of Oxygenated, Nitrated and Unsubstituted Polycyclic Aromatic Hydrocarbons Associated with Urban Aerosols*, *Mutation Research/Genetic Toxicology*, **371(3-4)**: 123-157 (1996).
- [35] Asante-Duah D.K., "Public Health Risk Assessment for Human Exposure to Chemicals". (Vol. 6). Kluwer Academic (2002).
- [36] FAO/WHO, Codex Alimentarius Commission., "Food Additives and Contaminants. Joint FAO/WHO Food Standards Programme, & World Health Organization". Codex Alimentarius: General Requirements (Food Hygiene). ALINORM 01/12A: 1-289 (2001).
- [37] Dimirezen D., Uruc K., *Comparative Study of Trace Elements in Certain Fish, Meat and Meat Products*. *Meat Science*, **74**: 255-260 (2006).
- [38] EC (European Commission)., "Setting maximum levels for Certain Contaminants in Foodstuffs, *Official Journal of the European Union* L364/5 Commission Regulation (EC) No 1881/2006 (2006).
- [39] Gopalani M., Shahare M., Ramteke D.S., Wate S.R., *Heavy Metal Content of Potato Chips and Biscuits from Nagpur City, India*. *Bull Environ Contam Toxicol*, **79(4)**: 384-387 (2007).
- [40] Iwegbue C.M., Nwozo S.O., Overah C.L., Bassey, F.I., Nwajei G.E., *Concentrations of Selected Metals in some Ready-to-eat-Foods Consumed in Southern Nigeria: Estimation of Dietary Intakes and Target Hazard Quotients*, *Turkish J. Agriculture-Food Sci. Tech.*, **1(1)**: 1-7 (2013).
- [41] Institute of Medicine., Food and Nutrition Board. "Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc". The National Academies Press, Washington, DC (2001).
- [42] NRC (National Research Council)., "Recommended Dietary Allowance", 10th ed., National Academy Press, Washington (1989).
- [43] WHO (World Health Organization) "Evaluations of the Joint FAO/WHO Expert Committee on Food Additives", <http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx?fcc=2>, Accessed January 23rd 2018(2017)
- [44] EVM (Export Group on Vitamins and Minerals) "Safe Upper Levels for Vitamins and Minerals of the Export Group on Vitamins and Minerals", Food Standard Agency (2003). <http://www.food.gov.uk/multimedia/pdfs/vitamin2003pdf>
- [45] Liu X., Song Q., Tang Y., Li W., Xu J., Wu J., Wang F., Brookes P.C., *Human Health Risk Assessment of Heavy Metals in Soil-Vegetable System: A Multi-Medium Analysis*, *Sci. Tot. Environ.*, **463**: 530-540 (2013).